



AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 1 following “CROSS REFERENCE TO RELATED APPLICATION(S)” with the following paragraph:

“This application is a divisional of U.S. Patent Application Serial No. 09/772,304, now U.S. Patent No. 6,756,222, filed January 29, 2001, which is incorporated herein by reference in its entirety.”

Please replace the paragraph starting at page 6, line 8 with the following paragraph:

“In an embodiment, the nucleic acid fragment coding for poly-beta-hydroxybutyrate synthesis pathway is a 4.826 Kb long fragment (SEQ ID NO:1).”

Please replace the paragraph starting at page 7, line 14 with the following paragraph:

“[[A]] As said earlier, polyhydroxybutyrate and other polyhydroxyalkanoate biosynthesis genes have been isolated, characterized and patented from organisms like *Ralstonia eutropha* (formerly known as *Alcaligenes eutrophus*), *Rhodospirillum rubrum*, *Zoogloea ramigera*. The Applicants have now have isolated, cloned, sequenced and characterized a 4.826 kb Sau3A I restriction endonuclease fragment from the genomic DNA of *Streptomyces aureofaciens* NRRL 2209 (SEQ ID NO:1) and this fragment carries all the genetic information required for the synthesis of polyhydroxybutyrate upon it being used to transform *Escherichia coli* JM109. This is the first instance of its kind where DNA fragment isolated from a member of actinomycetes has been expressed in a heterologous host for the production of polyhydroxybutyrate. The transformed *Escherichia coli* harboring the 4.826 kb Sau3A I restriction endonuclease fragment (SEQ ID NO:1) preferentially uses glycerol as a carbon source for the synthesis of polyhydroxybutyrate.

The putative polyhydroxybutyrate biosynthesis genes that the applicants have isolated does not show significant sequence similarity at amino acid level with any of the reported polyhydroxybutyrate biosynthesis gene sequences. While *Streptomyces aureofaciens* NRRL 2209 accumulates only about 1% polyhydroxybutyrate, the DNA fragment that the applicants have isolated from this organism and introduced into *Escherichia coli* JM109 supports production and accumulation of polyhydroxybutyrate to the extent of 60% of dry cell mass of this heterologous host. The novelty of the patent is the isolation, cloning, sequencing and heterologous statement of a 4.826 kb DNA sequence from *Streptomyces aureofaciens* NRRL2209 (SEQ ID NO:1) for the production of polyhydroxybutyrate.

Please replace the paragraph starting at page 8, line 15 with the following paragraph:

“The novelty of the invention resides in isolation, cloning, sequencing and characterization of a 4.826 kb Sau3A I restriction fragment from the genomic DNA of *Streptomyces aureofaciens* NRRL 2209 (SEQ ID NO:1). The Sau3A I DNA fragment (SEQ ID NO:1) harbors genes responsible for the synthesis of polyhydroxybutyrate. This Sau3A I DNA fragment (SEQ ID NO:1) when cloned and introduced into *Escherichia coli* JM109 as plasmid vector pSa240 supports the synthesis of polyhydroxybutyrate to the extent of at least 60% dry mass of the bacterial cell. The recombinant *Escherichia coli* JM109 (ATCC PTA-1579) utilizes glycerol as a carbon source for the synthesis of polyhydroxybutyrate.

Please replace the paragraph starting at page 10, line 4 with the following paragraph:

“**FIG. 8** represents the nucleotide sequence of the 4.826 kilobase Sau3A I genomic DNA fragment from *Streptomyces aureofaciens* NRRL2209 (SEQ ID NO:1) cloned and present as an insert in the pSa240 plasmid.”

Please replace the paragraph starting at page 24, line 5 with the following paragraph:

“The newly identified 4826 base pair Sau3A I restriction fragment from the genomic DNA of *Streptomyces aureofaciens* NRRL2209 (SEQ ID NO:1) which carries the genes for PHB synthesis is isolated and cloned.

Please replace the paragraph starting at page 24, line 8 with the following paragraph:

2. The newly identified 4826 base pair Sau3A I restriction fragment from the genomic DNA of *Streptomyces aureofaciens* NRRL2209 (SEQ ID NO:1) is cloned in a multicopy plasmid vector to create a new plasmid vector pSa240 which carries the nucleotide sequence of the PHB biosynthesis genes.”